



## EXHIBIT 1

KAROLINSKA INSTITUTET  
— a medical university

Department of	COLLEGE OF LIBRARIES
Laboratory Journal No.	1000
Name:	_____
Group:	_____
Date:	from _____ to _____

## Laboratory Journal

248909

cont. from page no.

Study

cont. on page no.

Project no.

Study no.

29

Assembly of a synthetic gene coding for  
Tel of 1 Chanc 1 using Tag

## PCR

Oligos 132, 133, 134, 135 10 μM

1 μl of each oligo 132-135

1 μl dNTP 10 mM

0.5 μl Tag

1 μl 10x Tag-buffer

3.5 μl H<sub>2</sub>O

10 μl

→ PCR Expedert program HANSI

99 °C 7 min

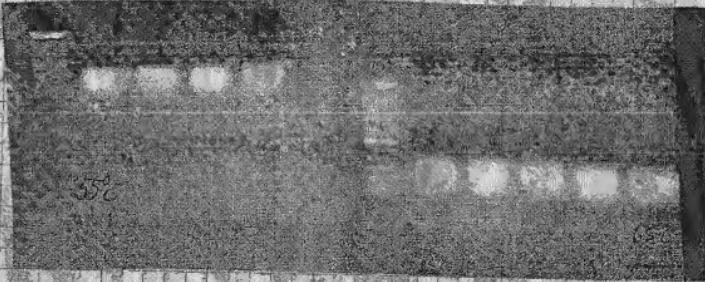
annealing 53-65 °C 15 min (grad)

elongation 68 °C 20 min

30 cycles end at 10 min elongation

+ 4 °C

Result A strong band around 300 (exp ~260)



Read and understood by



Laboratory Journal

248910

cont. from page no.

Study

cont. on page no.

Procedure

Study no.

Assembly of chain 2 Sel d1

primers 127-131 + 138

using 3 different DNA polymerases T4

Methyldesoxyribo nucleic acid



per

Ampli tag

excited band  
at 394

100

100

100

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## Laboratory Journal

248923

cont. on page no.

Study

Expression and purification of  
Tel d1 chain 1 and chain 2.

cont. on page no.

Project no.

Study no.

Tel d1 chain 1 (clone 42) and Tel d1 chain 2

clone 29 was ligated into pET 20b and  
electroporated into BL-21 DE3 cells after  
having been cut from pET7-Blue containing  
the correct sequence (see blinks HC1, Tel d1)

Sequencing of pET 42/pET 29 was done according  
to standard protocol (ABI) and the results  
can be seen on the opposite side.

Both Tel d1 chain 1 (Tel 1:1) and Tel d1 chain 2  
(Tel 1:2) was expressed according to standard  
protocol and purified on a thiophen (chelate)  
column loaded with NiSO<sub>4</sub>.

Chain 1 was soluble, after ultra sonication  
chain 1 was found in 20 mM Tris-HCl pH fraction,  
while chain 2 was found in the inclusion  
bodies after "washing" with 2M urea buffer + 20 mM  
Tris-HCl pH 6. The inclusion bodies were  
solubilized in 6M Guanidinium, transferred to  
6M urea buffer (20 mM Tris-HCl pH 6 + 0.5M NaCl)  
via 6 ml His-Harap. Purification was done on FPLC

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Date

Signature

Date

### Best service index (BSI) (Elliott 1993)

Parzellennummer	Fla. ha.	Fla. ha.	Abtrennung
340010-000-000	79,047	61,610	Abtrennung - - - - -
340010-010-000	1,000	1,000	Abtrennung - - - - -
340010-100-000	20,20	20,20	Abtrennung - - - - -
410010-000-000	33,500	33,500	Abtrennung - - - - -
410010-010-000	1,000	1,000	Abtrennung - - - - -
410010-100-000	10,743	10,743	Abtrennung - - - - -
430010-000-000	78,327	78,327	Abtrennung - - - - -
430010-010-000	78,327	78,327	Abtrennung - - - - -
470010-000-000	73,000	73,000	Abtrennung - - - - -
470010-010-000	73,000	73,000	Abtrennung - - - - -
470010-100-000	73,000	73,000	Abtrennung - - - - -
470110-000-000	39,000	39,000	Abtrennung - - - - -
470110-010-000	39,000	39,000	Abtrennung - - - - -
500010-000-000	20,000	20,000	Abtrennung - - - - -
500010-010-000	20,000	20,000	Abtrennung - - - - -
510010-000-000	22,000	22,000	Abtrennung - - - - -
510010-010-000	22,000	22,000	Abtrennung - - - - -
510010-100-000	22,000	22,000	Abtrennung - - - - -
580100-000-000	145,328	145,328	Abtrennung - - - - -
580100-010-000	145,328	145,328	Abtrennung - - - - -
580100-100-000	145,328	145,328	Abtrennung - - - - -
580200-000-000	152	152	Abtrennung - - - - -
580200-010-000	152	152	Abtrennung - - - - -
580200-100-000	152	152	Abtrennung - - - - -
580300-000-000	0,65	0,65	Abtrennung - - - - -
580300-010-000	0,65	0,65	Abtrennung - - - - -
580300-100-000	0,65	0,65	Abtrennung - - - - -
580400-000-000	146	146	Abtrennung - - - - -
580400-010-000	146	146	Abtrennung - - - - -
580400-100-000	146	146	Abtrennung - - - - -
580500-000-000	240	240	Abtrennung - - - - -
580500-010-000	240	240	Abtrennung - - - - -
580500-100-000	240	240	Abtrennung - - - - -
580600-000-000	380	380	Abtrennung - - - - -
580600-010-000	380	380	Abtrennung - - - - -
580600-100-000	380	380	Abtrennung - - - - -
580700-000-000	7,94	7,94	Abtrennung - - - - -
580700-010-000	7,94	7,94	Abtrennung - - - - -
580700-100-000	7,94	7,94	Abtrennung - - - - -
580800-000-000	0,76	0,76	Abtrennung - - - - -
580800-010-000	0,76	0,76	Abtrennung - - - - -
580800-100-000	0,76	0,76	Abtrennung - - - - -
580900-000-000	110	110	Abtrennung - - - - -
580900-010-000	110	110	Abtrennung - - - - -
580900-100-000	110	110	Abtrennung - - - - -
580200-015-000	150	150	Abtrennung - - - - -
600050-320-000	428	0,00	Abtrennung - - - - -
600050-330-000	428	0,00	Abtrennung - - - - -
600050-340-000	428	0,00	Abtrennung - - - - -
640050-000-000	0,83	0,83	Abtrennung - - - - -
640050-010-000	0,83	0,83	Abtrennung - - - - -
640050-100-000	0,83	0,83	Abtrennung - - - - -
740050-000-000	150	0,00	Abtrennung - - - - -
740050-010-000	150	0,00	Abtrennung - - - - -
740050-100-000	150	0,00	Abtrennung - - - - -

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0.47 11.4 265 265 13 83

11. *Leucanthemum vulgare* L. (Fig. 11) is a common species throughout the country. It is a low, spreading, hairy plant, 10-30 cm. high, with numerous branched, hairy, leafy stems. The leaves are deeply lobed, the lobes being narrow and pointed. The flowers are white, with yellow centers, and are arranged in corymbose clusters.

Urgent care for 32 min  
0.205 145 min

Raw data values (calculated):

	$\beta$	$\alpha$								
C	0.636	0.054	0.378	0.024	1.133	0.443	2.704	0.923	1.147	0.397
S	-2.321	0.216	0.485	0.105	0.162	0.770	0.917	0.418	0.179	0.303
SE	0.321	0.026	0.185	0.008	0.162	0.070	0.197	0.078	0.074	0.028

8. 0.024	0.173	0.088	0.032	0.011	0.011	0.011	0.011	0.011	0.043	0.179	0.086
9. 0.025	0.144	0.066	0.028	0.007	0.007	0.007	0.007	0.007	0.046	0.163	0.079

1.140 (1.154) 1.210 0.046 (0.30) 0.348 2.371  
 0.492 0.119 0.293 (0.154) 6.677 6.677 6.677 6.677

Conc: 100 mg/ml

6.  $\sqrt{17}$

1970-1971

11. *Leucosia* (Leucosia) *leucostoma* (Fabricius) (Fig. 11)

19. *Leucosia* *leucostoma* (Fabricius) (Fabricius, 1775: 400).  
19. *Leucosia* *leucostoma* (Fabricius) (Fabricius, 1775: 400).

1960-1961: The first year of the new program, the first year of the new school, the first year of the new curriculum.



## Laboratory Journal

248926

cont. on page no.  
Study no.cont. on page no.  
Projection

Study no.

Test of Fd 1 chain 1, clone 42 and  
Fd 1 chain 2, clone 29 with 6 cat sensitized  
Abopes from Algot study

6 felines from the Algot study, no 29, 59, 277, 422,  
434 and 454  
was diluted 13 times and 10 felines resp. in  
PBS, pH 7.4. A µ-filter plate was coated with:

## Plate 1

A	10 <sup>-2</sup> µg/ml chain 1	→	(all horizontal wells)
B	5 <sup>-2</sup> µg/ml "	→	
C	2.5 <sup>-2</sup> µg/ml "	→	
D	1.25 <sup>-2</sup> µg/ml "	→	
E	10 <sup>-2</sup> µg/ml clone 2	→	
F	5 <sup>-2</sup> "	→	
G	2.5 <sup>-2</sup> "	→	
H	1.25 <sup>-2</sup> "	→	

## Plate 2

A	5+5 µg/ml	chain 1+2 (a 1:1 mix of resp. chain)
B	2.5+2.5 "	chain 1+2 (1:1, 5 µg/ml each)
C	1.25+1.25 "	—

On the vertical rows the patients were added

1	2	3	4	5	6	7	8	9	10	11	12
↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓
Patient 29		59	277	422		434		454			
3x, 1ox	3x, 1ox										

## Laboratory Journal

248927

cont. from page no.

Study

cont. to page no.

Procedure

Study no.

(cont.)

ELISA conditions

Coating of El. 1st and El. 2nd over weekend in +4°C. Serum 4 times was with "Titer Counting" Patients serum was added, 100  $\mu$ l/well and incubated at +4°C o.n. Wash 4 times (Wallac Elast-washer) with "Titer Counting" and 100  $\mu$ l Rabbit human anti IgE dil 1000 times in "Var buffert" Incubation 2 hours in RT on shaker. Wash 4x "titration" and add 100  $\mu$ l/well of Goat anti-rabbit-ALP conjugated (DAKO) for 1h. Wash 4 times and add substrate 3 tablets/5ml of Var buffert.

The result was read in ELISA reader after 45 min at 405 nm

Result: 2.5  $\mu$ g IgE seems to be an adequate coating concentration for both chain 1 and chain 2. Mixing of the two chains can be done with coating concentrations 2.5 + 2.5  $\mu$ g/plate

Read and understood by

001114



1a 1b 2a 2b  
↓ ↓ ↓ ↓

Putting chain 1 and 2 (1+2)  
together (001121)

Template 1a 6ul  
Template done 29

1:10, 1:100, 1:1000

2ul

0.5ul primer (76  
2ul — 103  
2ul dNTP  
3ul 10X buff.  
1ul Pfu  
14.5ul H<sub>2</sub>O

30ul



1a 2b  
1:10 1:100

Result: One band  
of ~ 500 bp which  
should be chain 1+2

The bands are cut  
out and purified  
on Acrylamide

↓  
ligated with "perfectly linear"  
cloning kit. 10 colonies  
are picked for miniprep  
and possibly sequencing

(1a) 1st complete chain 1a, 42 (1:1000)

2ul primer 176

2ul — 174

2ul 10X buff

1ul Pfu

2ul dNTP (10mM)

10ul H<sub>2</sub>O

20ul

(1b) Some smear seen  
primer Tag polymerase

(2a) 1st temp. (1:1000) 10mM P<sub>1</sub>  
1st temp. ~ 10mM P<sub>2</sub>  
2ul primers 176, 174, 183  
10ul 1st smear (1a)

(2b) Some (2a) smear Tag

(3a) 1ul template bldg 2 (1000)  
2ul 179  
2ul 175  
10ul 1st smear 1a

(3b) Some (3a) smear Tag

(4a) 1ul template 1  
1ul template 2  
2ul primer 180  
10ul 1st smear (3a)

(4b) Some (4a) smear Tag

AmpliTaq Gold™  
250 Units, 50 μl  
Storage at -20°C

AD3912



## Laboratory Journal

248928

from [here](#).

www.mheducation.com

### Study

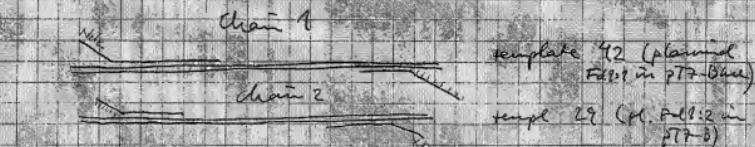
Linking of chain 1 and chain 2 (scamless)  
with PCR Del d19 and Del d22

Page 10

**Study no.**

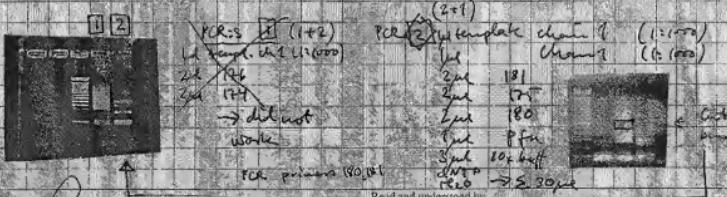
Aim: The aim of this experiment is to join the two chains of *Feb d1* into one construct by PCR

Study antibody The two sequenced chains of the major <sup>allergen</sup> ~~chains~~ of cat Fel d1 (chain 1, clone 42) and chain 2 (clone 29) is joined with PCR in two steps as outlined below. In



Result (see opposite side)

Good bands of expected size was seen for both chain 1 and chain 2. But (2a) + (2b) as well as (1a) and (1b) did not work. I will continue by adding (1a) to ~~template~~ 29 and to PCR. (Chain 1+2)



Reaff and understood by

### Significance

10

SINDBAUM

1000

ABI PRISM

Version 3.0

100

200

300

400

500

600

700

800

900

1000

1100

1200

1300

1400

1500

1600

1700

1800

1900

2000

2100

2200

2300

2400

2500

2600

2700

2800

2900

3000

3100

3200

3300

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3600

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4100

4200

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7200

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8600

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9100

9200

9300

9400

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9600

9700

9800

9900

10000

10100

10200

10300

10400

10500

10600

10700

10800

10900

11000

11100

11200

11300

11400

11500

11600

11700

11800

11900

12000

12100

12200

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30000

30100

30200

30300

30400

30500

30600

30700

30800

30900

31000

31100

31200

31300

31400

31



## Laboratory Journal

248931

cont. from page 204

Study Sequencing of 4 clones of each 1+2 and  
2+1

cont. on page no.

Project no.

Study no.

1+2 Clone no. Sig no.

Clone 1 = (1)  
2 = (2)  
3 = (3)  
9 = (9)

2+1

5 = (1) red - green  
6 = (2) yellow - green  
7 = (3) orange - green  
8 = (8)4.8  $\mu$ l reaction1.2  $\mu$ l primers9  $\mu$ l mix 80  $\mu$ l ethyl alcohol10 $\mu$ l+ 50  $\mu$ l oil and

PCR in Perkin Elmer

98°C 30

50°C 15 }

60°C 4 } 25 cycles

Unfortunately there was a scheduled power failure  
and the PCR-run was interrupted. <sup>about</sup> Assam + more  
cycles which is doneSamples are loaded on lanes 20 - 33 on ABI sequencer  
and named Fd 1 1+2 pt7 clone 4F- V - 4R  
- V - 5F  
- V - 5R  
etc.

Loading of clone 5 (1+2)

and clone 1 (2+1)

up in Hole 1 and 200  $\mu$ l

for lyophilization

20  $\mu$ l plasmid mix prep (pJ + BAC)2.4  $\mu$ l 10% w/v0.3  $\mu$ l BSA0.7  $\mu$ l Nuclei

Incentra 37°C shake

for 2 h.

bright  
pointLane clone  
1 (2+1) (1+2)

Read and understood by



## Laboratory Journal

248982

cont. from page no.

Study

Ligation of fd 1, clone 1 (1+) and clone 5 (1+) into pET 20b<sup>+</sup> and electroporation into BL21 phage

cont. on page no.

Project no.

Study no.

The fragments from fd 1 (1+) clone 1 and clone 5 (1+) were digested from 1% agarose gel (248931). A cleaned (Nde/Not) pET 20b<sup>+</sup> vector was used to ligate the fragments.

Conclusion

2 μl vector fragments  
10 μl vector  
15 μl 10 mM ATP  
1.8 μl 10% ligase buffer  
1.7 μl 1% ligase  
1.6 μl

Ligate + 16°C 60 min

The ligate mix was electroporated into 50 μl BL21-  
phage electrocompetent cells. 1 μl ligate mix  
was added to thawed cells (on ice). Electroporation  
according to standard protocol. Growth on SOC  
medium for 60' 37°C shaker (300 rpm) and  
plated on Amp/Can plates. One colony <sup>from</sup>  
each plate was picked and grown on LB Amp  
Can medium, mini prep (Chloram)  
and 25 μl of the (top) prep was  
run with Nde and Xba. Result ~ 800 bp  
Both clones contain the insert!!

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Date

Signature

Date



## Laboratory Journal

248933

Study: Protein Purification

Purification of Fd1 (1+2) clone 5 and  
Fd1 (2+1) clone 1 over Ni<sup>2+</sup> chelate Hi-TrapDept. or project no.  
Project no.

Study no.

1 Liter of Fd1 (1+2) and (2+1)<sup>1</sup> was grown to 0.6 (600 nm) and induced with 0.4 mM IPTG (see 248932)

Purification according to protocol. Both proteins were expressed as inclusion bodies and purified accordingly. Purification on FPLC as follow. After adsorption onto Ni-column in 6M GuH and wash also with 6M GuH the column is stuck to FPLC

Program:

0 conc % B0

0 ml/min 30 ml/min  
0 0.25 sulphur

0 post set 60

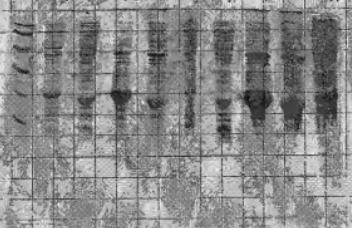
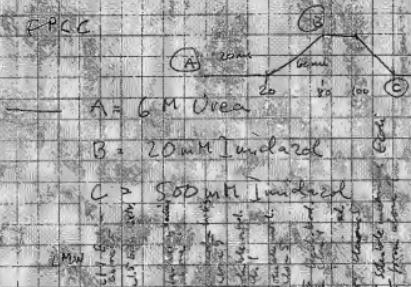
20 conc % B 0

30 conc % B 100

100 conc % B 100

125 conc % B 0

125 postset 60



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Signature

Date

Signature

Date